The heme regulatory motifs of heme oxygenase-2 function to transfer heme to the catalytic site for degradation

Angela S. Fleischhacker[ǂ], Amanda L. Gunawan[ǂ], Brent A. Kochert[§1], Liu Liu[ǂ], Thomas E. Wales[§], Maelyn C. Borowy[ǂ], John R. Engen[§], and Stephen W. Ragsdale[ǂ2]

From the [ǂ]Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48103 and [§]Department of Chemistry and Chemical Biology, Northeastern University, 360 Huntington Avenue, Boston, Massachusetts 02115

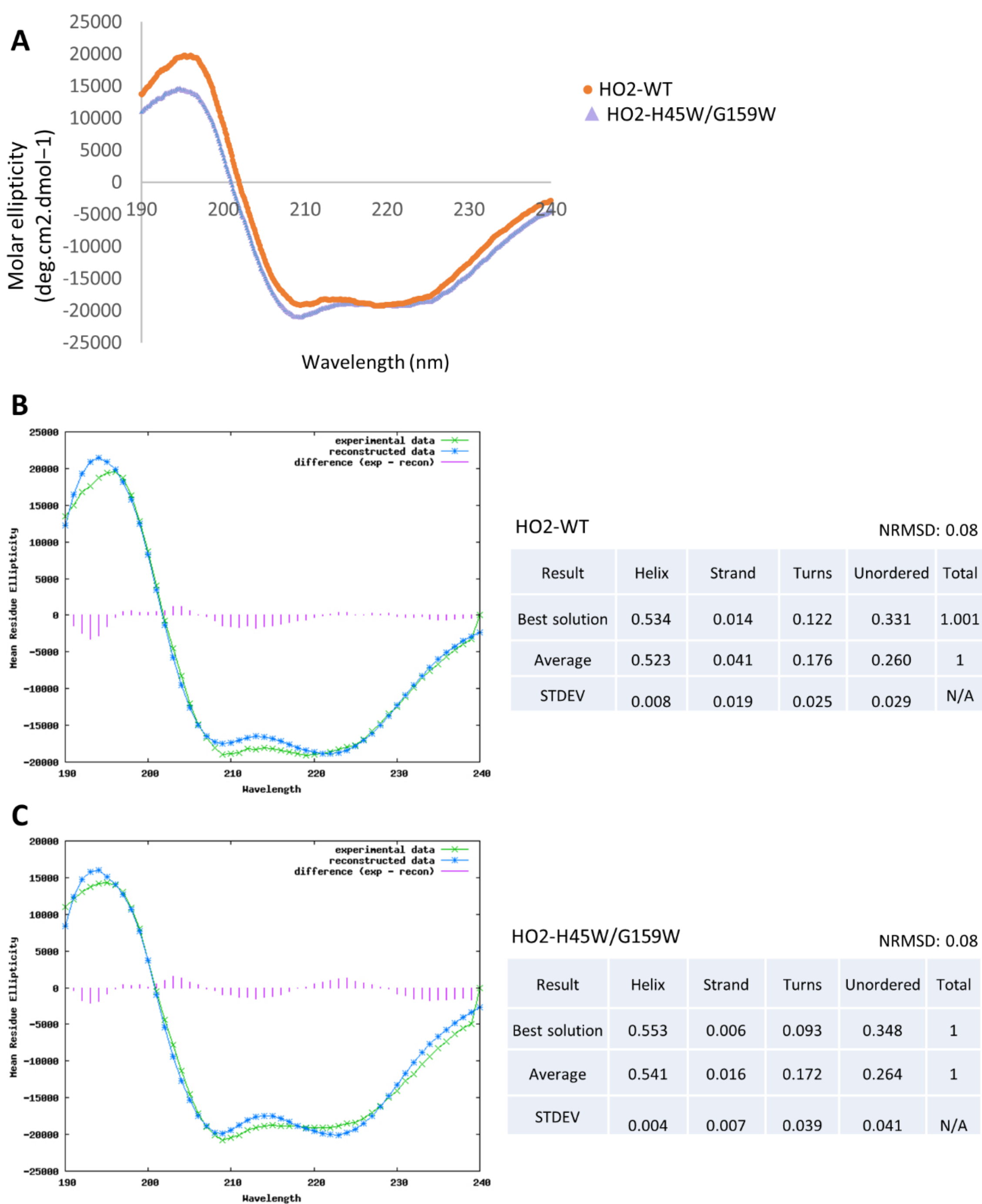
[1] Present address: AR&D Mass Spectrometry, Analytical R&D, Process R&D, Merck & Co., Inc., 2000 Galloping Hill Road, Kenilworth, NJ, 07033, USA
[2] To whom correspondence should be addressed: Department of Biological, Chemistry, University of Michigan Medical School, 1150 W. Medical Center Dr., 5301 MSRB III, Ann Arbor, MI USA, 48109-0606, Tel: 734-615-4621; Fax: 734-763-4581; E-mail: sragsdal@umich.edu

Supplemental Figures and Legends

Figure S1. Circular dichroism spectra of $HO2_{core}$ in 50 mM potassium phosphate pH 8.0.
Figure S2. Heme off-rate determination for G163H Fe^{3+}-$HO2_{core}$, as measured in an apo-H64Y/V68F-myoglobin assay
Figure S3. Heme transfer from HRM1 to the core after single turnover of heme in the core in the presence of biliverdin reductase.

Supplemental Excel Legend

Excel S1. Excel workbook containing all the measured relative deuterium values for all of the experiments. In each tab, the differences between the two states is shown at the right and corresponds to the difference map in the Figure indicated in the name of the tab.



HO2-WT

NRMSD: 0.08

Result	Helix	Strand	Turns	Unordered	Total
Best solution	0.534	0.014	0.122	0.331	1.001
Average	0.523	0.041	0.176	0.260	1
STDEV	0.008	0.019	0.025	0.029	N/A

HO2-H45W/G159W

NRMSD: 0.08

Result	Helix	Strand	Turns	Unordered	Total
Best solution	0.553	0.006	0.093	0.348	1
Average	0.541	0.016	0.172	0.264	1
STDEV	0.004	0.007	0.039	0.041	N/A

Figure S1. Circular dichroism spectra of $HO2_{core}$ in 50 mM potassium phosphate pH 8.0. *(A)* The spectra overlay of HO2-WT (orange) and the H45W/G159W mutant (purple), 5 µM in a 0.1 cm cell. *(B), (C)* Spectra of HO2-WT *(B)* and HO2-H45W/G159W *(C)* fit using CONTIN data base with DichroWeb server. The estimation of secondary structure is listed in the corresponding table.

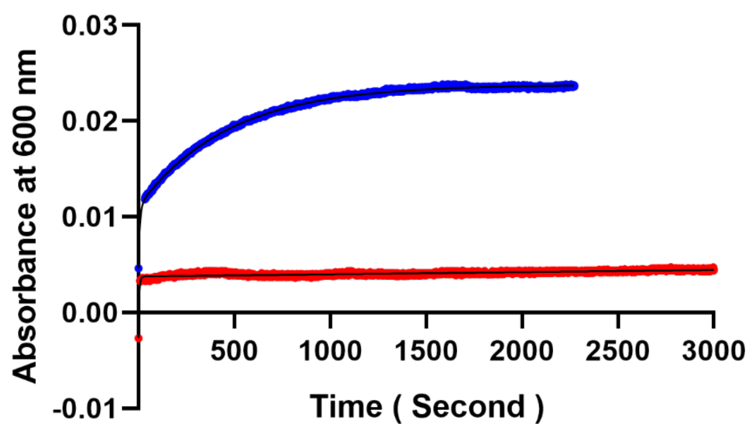
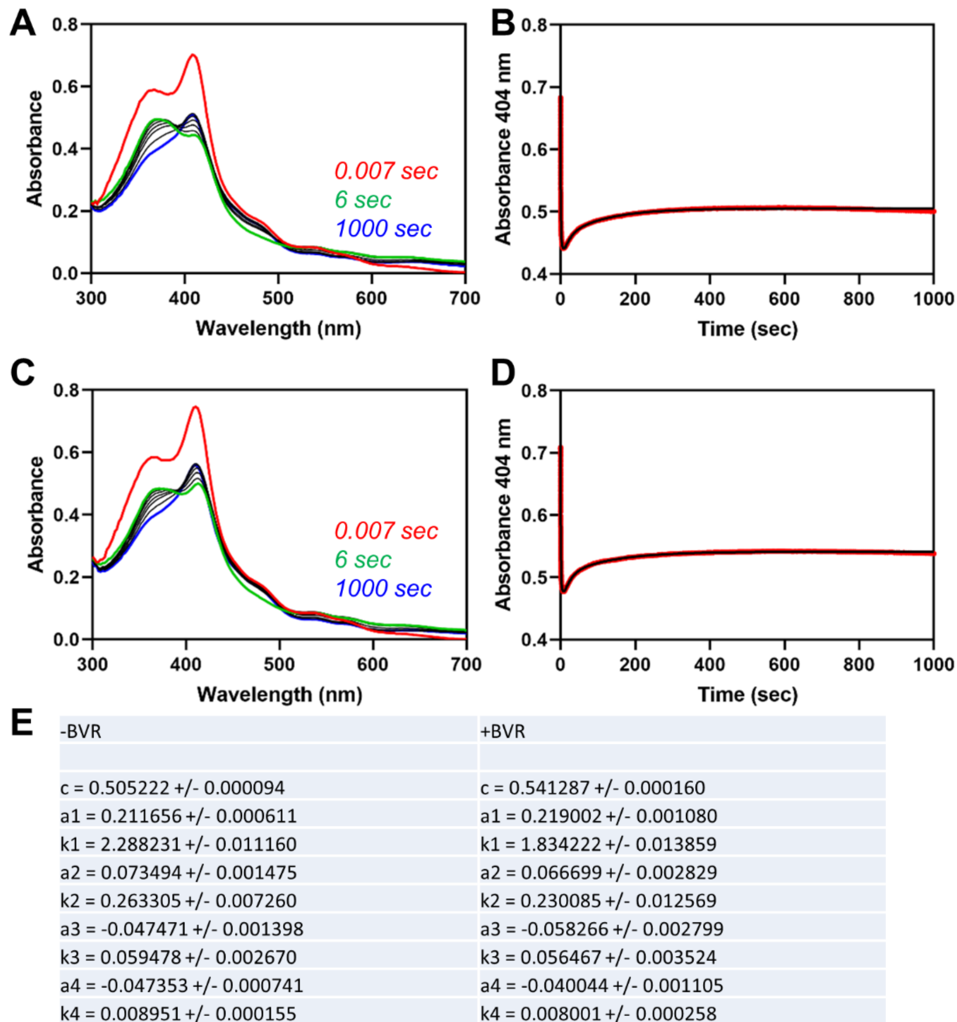


Figure S2. The heme off-rates for G163H Fe^{3+}-HO2*core*, as measured in an apo-H64Y/V68F-myoglobin assay. The absorbance change over time at 600 nm (red) is compared to the change in absorbance over time at 600 nm (blue) for wild-type Fe^{3+}-HO2*core* in the same assay. Fits of the data to a double exponential equation (black) are shown.



-BVR	+BVR
c = 0.505222 +/- 0.000094	c = 0.541287 +/- 0.000160
a1 = 0.211656 +/- 0.000611	a1 = 0.219002 +/- 0.001080
k1 = 2.288231 +/- 0.011160	k1 = 1.834222 +/- 0.013859
a2 = 0.073494 +/- 0.001475	a2 = 0.066699 +/- 0.002829
k2 = 0.263305 +/- 0.007260	k2 = 0.230085 +/- 0.012569
a3 = -0.047471 +/- 0.001398	a3 = -0.058266 +/- 0.002799
k3 = 0.059478 +/- 0.002670	k3 = 0.056467 +/- 0.003524
a4 = -0.047353 +/- 0.000741	a4 = -0.040044 +/- 0.001105
k4 = 0.008951 +/- 0.000155	k4 = 0.008001 +/- 0.000258

Figure S3. Heme transfer from HRM1 to the core after single turnover of heme in the core in the presence of biliverdin reductase. *A*, $Fe^{3+}_{core/HRM}$-HO2$_{sol}^{R}$ and an equimolar concentration of CPR were pre-mixed prior to being rapidly mixed with a limiting amount of NADPH to initiate degradation of heme at the core. The data at 0.007 seconds (red), 6 seconds (green), 1000 seconds (blue), and several representative intermediate time points (black) are shown. All measurements were carried out at 20 °C using the 1-cm path length configuration in photodiode array mode in triplicate. *B*, the stopped flow trace at the 404 nm (red) from one of the triplicates is shown. The data was fit to a quadruple exponential equation (black) using the Pro-data Viewer software provided by Applied Photophysics. *C and D*, same as *A* and *B*, respectively, except that an equimolar concentration of biliverdin reductase was added with HO2 and CPR prior initiating the reaction. *E*, fits of the traces shown in *B* and *D*.